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THU0230 **TNF- α PROMOTER POLYMORPHISMS (G-308A AND G-238A) ARE ASSOCIATED WITH SUSCEPTIBILITY TO SLE: A HOSPITAL-BASED CASE-CONTROL INVESTIGATION**

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Background: Tumour necrosis factor- α (TNF- α) is a proinflammatory cytokine associated with various autoimmune disorders¹. High levels of TNF- α has been reported in systemic lupus erythematosus (SLE)². Two functional common polymorphisms (G-238A and G-308A) at promoter region of TNF- α gene have been linked to SLE susceptibility in different populations^{3,4}.

Objectives: To investigate association of TNF- α (G-238A and G-308A) polymorphisms with susceptibility/resistance to SLE.

Methods: A total of 102 female SLE patients and 112 age and sex matched healthy controls were enrolled in the study. Patients were examined in detail, physical findings recorded and SLEDAI 2K calculated to assess disease severity. TNF- α polymorphisms (G-238A & G-308A) were typed by polymerase chain reaction and restriction length polymorphism (PCR-RFLP). Plasma level of TNF- α was quantified by ELISA. Statistical analysis was carried out using GRAPH PAD PRISM -7.01.

Results: Mean age of SLE patients and healthy controls was 27.84 $\pm$ 8.83 and 29.56 $\pm$ 5.48 years, respectively. At the time of enrolment, mean disease duration of patients was 2.07 $\pm$ 1.13 years. The mean SLEDAI 2K of patients was 16.07 $\pm$ 7.66. The prevalence of heterozygous mutant and minor allele of TNF- α (G-238A) polymorphisms were significantly higher in SLE patients compared to healthy controls (GA: P=0.04, OR=2.16; A: P=0.02, OR: 2.09). Furthermore, heterozygous (GA) and minor allele (A) of TNF- α (G-238A) polymorphism were associated with susceptibility to lupus nephritis (GA: P=0.02, OR=2.89; A: P=0.001, OR: 2.92). SLE patients displayed higher levels of plasma TNF- α compared to healthy controls. Although the prevalence of heterozygous mutant and minor allele of TNF- α (G-308A) polymorphism was higher in SLE patients, it was not statistically significant. TNF- α (G-238A and G-308A) variants were associated with higher plasma TNF- α in both SLE patients and healthy control. However, no significant association was observed on distribution of TNF- α polymorphisms (G-238A and G-308A) with severity of disease activity of SLE patients.

Conclusion: The results of the present study demonstrate that TNF- α (G-238A) variant is associated with higher plasma TNF- α level and increased susceptibility to development of SLE and lupus nephritis.

REFERENCES:

- [1] Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood*. 2012;119(3):651–665.
- [2] Studnicka-Benke A, Steiner G, Petera P, Smolen JS. Tumour necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. *Br J Rheumatol*. 1996;35(11):1067–1074.
- [3] D'Alfonso S, Colombo G, Della Bella S, Scorza R, Momigliano-Richiardi P. Association between polymorphisms in the TNF region and systemic lupus erythematosus in the Italian population. *Tissue Antigens* 1996;47(6):551–555.
- [4] Lee YH, Harley JB, Nath SK. Meta-analysis of TNF-alpha promoter-308 A/G polymorphism and SLE susceptibility. *Eur J Hum Genet*. 2006;14:364–371.

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THU0231 **GELSOLIN A NEW BIOMARKER OF DISEASE ACTIVITY IN SLE PATIENTS ASSOCIATED WITH HDL-C**

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Background: Recent proteomics techniques have demonstrated that high-density lipoprotein (HDL) associated proteins are involved in functions related to systemic inflammatory and immune responses in several pathological conditions, including autoimmune diseases. HDL undergoes structural and functional modifications in systemic lupus erythematosus (SLE) patients.

Objectives: To identify potential biomarkers of disease activity analyzing the proteome of HDL particles from SLE patients in clinical remission and when they develop a flare compared to a healthy control group.

Methods: Quantitative proteomic analyses of purified HDL were performed using Tandem Mass Tag (TMT) isobaric tag-labeling and nanoLC-Orbitrap (nLC-MS/MS) from 9 SLE patients in clinical remission when they developed a flare and from 9 healthy controls (9-9-9). We verified the identified proteins by Western blot and ELISA in a cohort of 104 SLE women patients, 46 healthy women and 14 SLE patients when developed a flare.

Results: A total of 83 proteins associated with HDL were identified. We found 17 proteins with a significant fold-change (>1.1) compared with their levels in control patients. In lupus patients experiencing a flare compared with those in remission, we identified 4 proteins with a significant fold-change (C4, Indian Hedgehog protein, S100A8 and gelsolin). Plasma Gelsolin (pGSN) levels were decreased in the 104 SLE patients (176.02 (74.9) mcg/l) compared with the control group (217.13(86.7)mcg/l); p = 0.005 and when they developed a clinical flare (104.84(41.7)mcg/l); p = 0.002). pGSN levels were associated with HDL-c levels (r = 0.316, p < 0.001). Antimalarial treated patients showed significant higher levels of pGSN (214.56(88.94)mcg/l respect 170.35(66.36) mcg/l); p = 0.017.

Conclusion: The proteome cargo from HDL differentiates SLE patients from healthy controls. HDL from SLE patients carries proteins that are involved in the activation of the immune system. Decreased pGSN are associated with clinical disease activity in SLE patients. Antimalarial treatment and HDL-c are associated with higher levels of pGSN in SLE patients. pGSN is a potential biomarker of disease activity in SLE patients.

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THU0232 **DIFFERENTIAL METHYLATION OF IL8 AND TISSUE FACTOR PROMOTER IN ANTIPHOSPHOLIPID SYNDROME**

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Background: Antiphospholipid syndrome (APS) is an autoimmune thrombophilia characterized by recurrent thromboembolism and/or pregnancy morbidity in the presence of Antiphospholipid antibodies mainly anti- β 2glycoprotein I (anti- β 2GPI), which lead to monocyte and endothelial cell activation and subsequent tissue factor and proinflammatory cytokine expression such as IL-6 and IL-8 (1). Epigenetics describes changes in gene expression without alterations in the genomic sequence. Methylation of DNA at CpG islands by adding a methyl group to the nucleotide cytosine, is one of main epigenetic mechanisms.

Objectives: To explore the possible differential methylation of IL8 and Tissue Factor (F3) gene promoters, which are critical for the pathophysiology of APS.

Methods: Whole blood and serum were isolated from 27 APS patients and 25 healthy donors (HDs). Human umbilical vein endothelial cells (HUVECs) and peripheral blood monocytes were isolated from 3 HDs. Anti- β 2GPI IgG was isolated and pooled from 8 APS patients. HUVECs and monocytes were stimulated with a mixture of IgG, β 2GPI and CXCL4. Then mRNA was isolated and qPCR was performed for the assessment of IL8 and tissue factor (F3) gene expression. Whole blood DNA from APS patients and HDs, and DNA from the *in vitro* experiments was isolated and bisulphite treated. The methylation of 1CpG in the

promoter of F3 gene and 1CpG in the promoter of IL8 gene was assessed with Methylation Specific PCR

Results: DNA from APS patients had significantly lower methylation levels in the IL8 promoter compared to HDs (mean: APS 0,6715vs HD1,153, $p < 0,0001$). Patients with history of arterial thromboembolism had the lowest IL8 methylation levels compared to ones with history of venous thrombosis or pregnancy morbidity, $p=0.0003$. An inverse correlation was noted between the IL8 methylation levels and the time passed since the last clinical event. APS patients who suffered 3 or more clinical incidents had significantly lower methylation levels.

APS DNA was significantly methylated in the F3 promoter compared to HD (APS 1,902vs HD1,335, $p<0,0001$). Negative association of F3 methylation was noted with anti- β 2GPI, auto-antibody titers and APTT elongation.

HD HUVECs and monocytes stimulated with anti- β 2GPI IgG/ β 2GPI/CXCL4 obtained an activated and procoagulant phenotype. In HUVECs the triple mixture induced an 8fold increase for the SELE gene, 3fold for the IL8, 1.5fold for F3. At the same time 2-fold decreased F3 promoter methylation levels correlated inversely to F3 gene expression ($r = -0.7679$, $p=0.0008$). No change was noted in the IL8 promoter methylation levels. Treated monocytes had a 40fold increase in the IL8 and 20 fold increase in the F3 gene levels, while methylation of IL-8 and F3 promoter regions was decreased by 2-fold.

HUVECs stimulation resulted in the differential expression of genes involved in DNA epigenetic alterations. Significant difference was observed for MeCP2 (2fold), DNMT3B (2 fold) and HDAC9 genes (3fold), which correlate with the expression of activation markers of SELE, F3 and IL8.

Conclusion: APS patients expressed decreased relative IL8 methylation levels which associate with arterial clinical events and significantly with more recent events. The mixture of anti- β 2GPI IgG- β 2GPI-CXCL4 activate HD HUVECs and monocytes to a procoagulant phenotype and decrease methylation in the F3 and IL8 promoters.

REFERENCES:

- [1] Allen, K.L., et al., A novel pathway for human endothelial cell activation by antiphospholipid/anti-beta2 glycoprotein I antibodies. *Blood*, 2012. 119(3): p. 884-93.

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THU0233 ASSOCIATION BETWEEN DEMYELINATING DISEASE AND AUTOIMMUNE RHEUMATIC DISEASE. A MULTIDISCIPLINARY EVALUATION

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Background: Multiple sclerosis (MS) and neuromyelitis optica (NMO) are autoimmune disease which target is the myelina of the white substance of CNS. Presents alterations of the white substance observed in MRI, like hyperintense injuries in T2, in brain and/or the spinal cord. These injuries are unspecific; they can appear as regards to arterial hypertension or diabetes, thus the MS diagnosis will be established according to criteria of location and course of these injuries. The systemic autoimmune rheumatic diseases (SARD) may be presented by demyelination areas in the white matter of the brain and spinal cord, which are difficult to differentiate from MS and other demyelinating processes; but although most of CNS pathologic of the SARD are small vessels disease. On the other hand, it has been described in the MS positividad for ANA, antiphospholipid antibodies (aPL) and Anti-Ro52 without SARD evidences that there might reflect a disregulación of the immune system or a form fruste of associate SARD.

Objectives: 1.To describe the SARD predominance in patients whose the guide sign is the presence of injuries desmielinizantes in CNS. 2 To describe the clinical profile of these patients. 3. To describe the frequency of reumatic antibody positivity in MS.

Methods: Retrospective observational study of patients with CNS demyelinating lesions as a guide sign and clinical-analytical findings suggestive of SARD assessed jointly by the Demyelinating Diseases Unit and and by the Rheumatology Service. MS diagnosis according to McDonald 2010 criteria. Statistical analysis with SPSS 17.0. carried out in the conditions of the usual clinical practice in the Consultations of Rheumatology and Neurology. We analyzed the patients referred from Consultations of Rheumatology to Neurology by finding demyelinating lesions in imaging test or

suggestive neurological symptoms. And patients referred fro Consultations of Neurology to Rheumatology by finding clinical or analytical signs of systemic autoimmune rheumatologic disease during the study of a demyelinating lesion. We included patients assessed in Consultations of both services from 1/1/2010 to 31/12/2017.

Results: Of 561 patients evaluated in Demyelinating Diseases Unit, 89 were evaluated also in Rheumatology. 82% were women, with average age of start the neurological symptoms at 40.6 years (SD 13.15), the average age at the time of assessment by Rheumatology 42.9 years (SD 12.8). Only 17 (19%) had previous a rheumatology diagnosis: Fibromyalgia 5 (5.6%); SARD 9 (10%). A definitive diagnosis was made in 63 patients (71%), probable 14 (16%) and nonspecific 12 (13.5%). Definitive diagnoses include: MS 32 (35.9%), NMO 4 (4.5%) and SARD 32 (35.9%); Neurosarcoidosis 3, NeuroSjögren 6, NeuroBeçet 2, Neurolupus 3, aPLS 7 and in the context of UCTD 11. First neurological symptom in the SARD was sensitive deficit 11 (22%). SARD was ruled out in 50 patients (fibromyalgia or degenerative pathology). Patients with definitive diagnosis of MS (32) and positivity for antibodies: ANA requested in 29 patients; positive 46.6%, Anti-Ro52 requested in 28 and all negative.

Conclusion: 1.The multidisciplinary assessment allowed to establish a definitive diagnosis in 71% of the patients. It allowed the new diagnosis of SARD in 55% of patients. 2. The 22% of SARD presented a sensitive deficit as the first neurological symptom. The main symptom suggestive of SARD that manifested the patients with demyelinating lesions were the inflammatory arthralgias, second probable Raynaud phenomenon and third ocular dryness.3. The 46.6% of MS have ANA positive without another sign of SARD.

Distinguishing between an MS and SARD with neurological manifestations is a diagnostic challenge.

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THU0234 THE TRANSCRIPTION FACTORS IKAROS (IKZF1) AND AIOLIS (IKZF3) ARE EXPRESSED IN THE SALIVARY GLANDS OF SJÖGREN'S SYNDROME PATIENTS WITH LYMPHOID AGGREGATES AND MODULATE B CELL ACTIVATION IN VITRO

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Background: Polymorphisms of Ikaros (IKZF1) and Aiolos (IKZF3), transcription factors essential for the maturation, differentiation and survival of B cells, have been linked to systemic autoimmunity. The Cereblon modulator iberdomide, known to induce their degradation, is being explored as a therapeutic option in Systemic Lupus Erythematosus (SLE). However, the involvement of Ikaros and Aiolos in other autoimmune diseases is poorly known, and their effects on B cell activation and differentiation in the context of autoimmunity have been only partially described.

Objectives: To evaluate the expression of Ikaros and Aiolos in the salivary glands (SGs) of patients with Sjögren's syndrome (SS) and the effects of iberdomide on the TLR7-mediated activation of B cells from patients with Sjögren's.

Methods: Immunohistochemical staining for Ikaros and Aiolos was performed on SG biopsies of patients with SS (n=23), classified into Ectopic Lymphoid Structures (ELS) positive (n=12) and negative (n=11) according to the presence of infiltrating B cells (CD20) and T cells (CD3), assessed by immunohistochemistry. SGs of patients with non specific chronic sialoadenitis (NSCS) (n=12) were used as controls. Digital image analysis and automated cell count (QuPath software) were used to calculate the density of Ikaros and Aiolos positive cells, which were correlated with histological and serological markers (SG semiquantitative scores for CD3+ T cells, CD20+ B cells, CD138+ plasmacells; Immunoglobulins; C3, C4) and disease activity (ESSDAI). CD19+ B cells were isolated from the peripheral blood of patients with Sjögren's (n=7) and triggered with the TLR7 ligand Resiquimod, with or without iberdomide (100 nM). After 5 days, cells were analysed by FACS and IgG and IgM in the supernatants were measured by ELISA.